

Claims

1. Modified sarcosine oxidase which is a protein converted by adding, deleting, inserting or substituting at least one amino acid in an amino acid sequence which constitutes a protein having
5 a sarcosine oxidase activity, characterized by having the sarcosine oxidase activity and having improved stability in a liquid compared one before conversion.
2. The modified sarcosine oxidase according to claim 1 characterized in that at least one amino acid in the amino acid
10 sequence which constitutes the protein having the sarcosine oxidase activity is substituted with other amino acid.
3. The modified sarcosine oxidase according to claim 1 wherein the protein having the sarcosine oxidase activity has homology of at least 50% or more to an amino acid sequence of SEQ ID NO:1.
- 15 4. The modified sarcosine oxidase according to claim 1 wherein the protein having the sarcosine oxidase activity has homology of at least 80% or more to the amino acid sequence of SEQ ID NO:1.
5. The modified sarcosine oxidase according to claim 1 wherein the protein having the sarcosine oxidase activity has the amino
20 acid sequence of SEQ ID NO:1.
6. The modified sarcosine oxidase according to claim 1 characterized in that at least one amino acid in a region corresponding to positions 155 to 250 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.
- 25 7. The modified sarcosine oxidase according to claim 1 characterized in that at least one amino acid in a region corresponding to positions 82 to 92 or 354 to 366 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.
8. The modified sarcosine oxidase according to claim 1
30 characterized in that at least one amino acid selected from the group consisting of a region corresponding to positions 89, 155, 166, 204, 213, 233, 240, 250 and 364 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.
9. The modified sarcosine oxidase according to claim 1
35 characterized in that lysine at position 89 is substituted with

arginine in the amino acid sequence of SEQ ID NO:1.

10. The modified sarcosine oxidase according to claim 1 characterized in that cysteine at position 155 is substituted with isoleucine in the amino acid sequence of SEQ ID NO:1.

5 11. The modified sarcosine oxidase according to claim 1 characterized in that asparagine at position 166 is substituted with lysine in the amino acid sequence of SEQ ID NO:1.

12. The modified sarcosine oxidase according to claim 1 characterized in that methionine at position 204 is substituted with alanine in the amino acid sequence of SEQ ID NO:1.

13. The modified sarcosine oxidase according to claim 1 characterized in that serine at position 213 is substituted with proline in the amino acid sequence of SEQ ID NO:1.

14. The modified sarcosine oxidase according to claim 1 characterized in that cysteine at position 233 is substituted with serine in the amino acid sequence of SEQ ID NO:1.

15. The modified sarcosine oxidase according to claim 1 characterized in that asparagine at position 240 is substituted with tyrosine in the amino acid sequence of SEQ ID NO:1.

20 16. The modified sarcosine oxidase according to claim 1 characterized in that glutamic acid at position 250 is substituted with glutamine in the amino acid sequence of SEQ ID NO:1.

17. The modified sarcosine oxidase according to claim 1 characterized in that alanine at position 364 is substituted with valine in the amino acid sequence of SEQ ID NO:1.

18. Modified sarcosine oxidase which is a protein converted by adding, deleting, inserting or substituting at least one amino acid in an amino acid sequence which constitutes a protein having a sarcosine oxidase activity, characterized by having the sarcosine oxidase activity and having a lowered action on L-proline compared with one before conversion.

19. The modified sarcosine oxidase according to claim 18 characterized in that at least one amino acid is substituted with other amino acid in the amino acid sequence which constitutes the

protein having the sarcosine oxidase activity.

20. The modified sarcosine oxidase according to claim 18 wherein the protein having the sarcosine oxidase activity has homology of at least 50% or more to an amino acid sequence of SEQ ID NO:1.

21. The modified sarcosine oxidase according to claim 18 wherein the protein having the sarcosine oxidase activity has homology of at least 80% or more to the amino acid sequence of SEQ ID NO:1.

22. The modified sarcosine oxidase according to claim 18 wherein the protein having the sarcosine oxidase activity has the amino acid sequence of SEQ ID NO:1.

23. The modified sarcosine oxidase according to claim 18 characterized in that at least one amino acid at positions 82 to 152 or 216 to 328 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.

24. The modified sarcosine oxidase according to claim 18 characterized in that at least one amino acid at positions 82 to 97 or 313 to 328 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.

25. The modified sarcosine oxidase according to claim 18 characterized in that at least one amino acid selected from the group consisting of positions 89, 94, and 322 in the amino acid sequence of SEQ ID NO:1 is substituted with other amino acid.

26. The modified sarcosine oxidase according to claim 18 characterized in that lysine at position 89 is substituted with arginine in the amino acid sequence of SEQ ID NO:1.

27. The modified sarcosine oxidase according to claim 18 characterized in that valine at position 94 is substituted with glycine in the amino acid sequence of SEQ ID NO:1.

28. The modified sarcosine oxidase according to claim 18 characterized in that lysine at position 322 is substituted with arginine in the amino acid sequence of SEQ ID NO:1.

29. The modified sarcosine oxidase according to claim 18 characterized in that a Km value for sarcosine after the

modification is within 3 times compared with unmodified one.

30. The modified sarcosine oxidase according to claim 18 characterized in that a Km value for sarcosine after the modification is within 1.5 times compared with unmodified one.

5 31. Sarcosine oxidase having at least one property of the followings under a measurement condition at 37°C and pH 8.0:
action on L-proline: 0.7% or less based on sarcosine, and
Km value for L-proline: 150 mM or more.

32. The sarcosine oxidase according to claim 31 characterized
10 by having at least one property of the followings under the measurement condition at 37°C and pH 8.0:

action on L-proline: 0.5% or less based on sarcosine, and
Km value for L-proline: 200 mM or more.

33. The sarcosine oxidase according to claim 31 wherein the Km
15 value for sarcosine is 10 mM or less.

34. The sarcosine oxidase according to claim 31 wherein the Km value for sarcosine is 5 mM or less.

35. A gene encoding the modified sarcosine oxidase according to any one of claims 1 to 17 and 18 to 30.

20 36. A vector containing the gene according to claim 35.

37. A transformant transformed with the vector according to claim 36.

38. A process for producing modified sarcosine oxidase characterized in that the transformant according to claim 37 is
25 cultured and sarcosine oxidase is collected from the culture.

39. A process for producing modified sarcosine oxidase which is excellent in substrate specificity, characterized in that a microorganism capable of producing the sarcosine oxidase according to any one of claims 31 to 34 is cultured and sarcosine
30 oxidase is collected from the culture.

40. A reagent for measuring creatine containing the sarcosine oxidase according to any one of claims 1 to 17, 18 to 30, and 31 to 34.

41. A reagent for measuring creatinine containing the sarcosine
35 oxidase according to any one of claims 1 to 17, 18 to 30 and 31

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to 34.